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A Phase III Randomised Trial Of Chemo-Immunotherapy Comprising Gemcitabine And Capecitabine (GemCap) With Or Without Telomerase Peptide Vaccine GV1001 In Patients With Locally Advanced Or Metastatic Pancreatic Cancer: Final Results Of The TeloVacTrial.

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SUMMARY

Background. This study tested the survival efficacy in advanced pancreatic cancer of telomerase vaccination (GV1001) in combination with sequential or simultaneous chemotherapy.

Methods. TeloVac was a three-arm, randomised phase III trial in locally advanced/metastatic pancreatic cancer and is registered as an International Standard Randomised Controlled Trial (ISRCTN4382138). The primary end-point was to investigate the efficacy of GV1001 on length of survival when added concurrently or sequentially to the combination of gemcitabine and capecitabine (target=1110 patients; 2-sided α=0·025 for each comparison with control). Eligible patients were treatment naïve, aged >18 years, with locally advanced or metastatic pancreatic ductal adenocarcinoma, and performance status of 0-2. Chemotherapy comprised six cycles of gemcitabine (1000mg/m^{2}30min intravenous infusion, days 1, 8, and 15) and capecitabine (830 mg/m^2 orally twice daily for 21 days, repeated every 28 days). Sequential chemoimmunotherapycomprised two cycles of combination chemotherapy,then an intradermal lower abdominal injection of granulocyte-macrophage colony-stimulating factor (75μg), and GV1001 (0·56 mg) (days 1, 3, and 5, once on weeks2-4, and 6 monthly thereafter). Concurrent chemoimmunotherapycomprisedgiving GV1001 from the start of chemotherapy. Treatments were allocated with equal probability by means of computer-generated random permuted blocks of sizes 3 and 6 in equal proportion. Analysis was by intention to treat.

Findings. The final results show that the median overall survival times for the chemotherapy (n=358), sequential chemoimmunotherapy (n=350) and the concurrent chemoimmunotherapy (n=354) arms were 7.89 (95% CI: 7.07- 8.85), 6.94 (95% CI 6.35-7.6) and 8.36 (95% CI: 7.30 -9.74) months respectively. The corresponding hazard ratios for the chemoimmunotherapy arms were 1.19 (98.25% CI: 0.97-1.48, p=0.047) and 1.05 (98.25% CI: 0.85-1.29, p=0.64),

with an overall log-rank χ^2_{2df}= 4.3; P=0•11). The corresponding median times to progression were 6.35 (95% CI: 4.77- 7.07), 4.54 (95% CI 4.34-4.61) and 6.58 (95% CI: 5.03 -7.27) months, with corresponding hazard ratios for the chemoimmunotherapy arms of 1.50 (98.25% CI: 1.26-1.78, p<0.001) and 1.0 (98.25% CI: 0.84-1.19, p=0.99) with an overall log-rank χ^2_{2df} = 29·5; P<0•001). Delayed type hypersensitivity was positive in 19 (12·3%) of 154 and 47 (20·2%) of 233 patients with sequential and concurrent chemoimmunotherapyrespectively (P=0·053).

The commonest grade 3/4 toxicities were neutropenia that occurred in 68 (19 %), 58 (17 %) and 79 (22 %) patients (X^2_{2df}=3·76; p=0·15) in the chemotherapy, sequential chemoimmunotherapy and the concurrent chemoimmunotherapy arms respectively; fatigue in 27 (7%), 36 (10%) and 44 (12%) patents respectively (X^2_{2df}=4·72; p=0·094); and pain in 34 (9%), 39 (11%) and 42 (11%) patients respectively (X^2_{2df}=1·09; p=0·58).

Interpretation. Adding GV1001 vaccination to chemotherapy did not improve survival. Stopping chemotherapy after two cycles was associated with reduced time to progression and suggests that treatment until progression is the appropriate approach in pancreatic cancer. Vaccination to telomerase can elicit immune responses during chemotherapy but without clinical efficacy but could be overcome by newer strategies.

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INTRODUCTION

The outcome of patients with advanced pancreatic cancer remains poor.[1]The median survival for those with metastatic disease is below 12 months even when treated with the most active chemotherapyregimens.[2,3]Radiotherapy appears to add little to the management of those with unresectable locally advanced disease.[4]Thus, more effective therapeutic strategies are required with immunotherapy being a most promising approach.[5,6]

During repeated rounds of DNA replication the telomeric ends of DNA become progressively shortened and without a compensatory mechanism cells senesce and die.[7,8]Reactivation of telomerase is a crucial event in transformation and occurs in nearly all pancreatic cancers.[9]GV1001 is a human telomerase reverse transcriptase catalytic subunit (hTERT) class II 16mer peptide vaccine .[7,8]A phase II trial of GV1001 in advanced pancreatic cancer showeda total immune response in 24 (63.2%) of 38 patients providing agreater median survival in immune responders of 216 days compared to 88 days for non-responders.[10] Patients treated with the vaccine doses and schedule applied in the current trial, had the highestimmune response (75%) .[10] GV1001 vaccinationhas been shown to generate multiple CD4+ clones that recognised naturally processed hTERT generating fragments fitting into multiple class II molecules. The clones also recognised T cell-depleted mononuclear cells from a patient with malignant ascites. Studies in other cancer patients have shown that GV1001 induces CD8+ cytotoxic T cells and can initiate epitope spreading.[10-13]

Although cytotoxicsare generally regarded as immunosuppressive, certain chemotherapy regimens may potentiate the effect ofcancer vaccines.[14-19]Gemcitabine and 5-fluorouracil induce apoptosis of cancer cells leading to the release of antigens which can be taken up by

professional antigen presenting cells and cross-presented to prime cytotoxic T-lymphocytes.[14,20]Ligation ofCD40 on antigen presenting cells withCD40L present on activated CD4+ cells determines the generation of cytotoxic T-lymphocytes.GV1001 vaccination was expected to generate telomerase-specific T helper cells in order to activate multipleantigen presenting cells loaded with diverse antigens,to prime and activate cytotoxic T-lymphocytes with a broad repertoire. Synergy between CD40 ligation and gemcitabine is maximal when gemcitabine is given prior to CD40 ligation.[21] Chemotherapy delivered after immunotherapy can enhance the effect of immunotherapy by delivering a bolus of tumour antigens and immunostimulatory signals.[15]Thus pre-clinical studies had clearly shown synergy between gemcitabine and both CD40 agonism and vaccines in certain cancer models. [14,15,21]

The combination of gemcitabine and capecitabine (an orally active 5-fluorouracil pro-drug) is a standard of care with improved objective response and time to progression compared to gemcitabine montherapy.[22] The design of the TeloVac study was based on the clear clinical evidence of immunogenicity of GV1001 in patients with pancreatic cancer, the available pre-clinical data demonstrating the synergy of gemcitabine with cancer vaccines and the other positive immunomodulatory effects of gemcitabine and fluoropyrimidines. Thus the TeloVac study aimed to exploit the positive immunomodulatory effects of these agentsand tested the impact of combining GV1001 with granulocyte-macrophage colony-stimulating factor (GM-CSF) as an adjuvant.[23]

METHODS

Study Design and Participants

TeloVac was a multicentre, three-arm, parallel group, open-label, phase III randomised controlled trial conducted at 51 United Kingdom sites. Eligible patients were treatment naïve, aged >18 years, with histologically or cytologically confirmed locally advanced or metastatic pancreatic ductal adenocarcinoma, an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 and adequate end organ function. Other specific inclusion criteria were locally advanced or metastatic disease precluding curative surgical resection or patients who have relapsed following previously resected pancreatic cancer; contrast enhanced computed tomography (CT) scan of the thorax, abdomen and pelvis within 28 days prior to commencing treatment; unidimensionally measurable disease on CT in accordance with the RECIST guidelines; platelet count $\geq 100 \times 10^9$/L, white blood cell count (WBC) $\geq 3 \times 10^9$/L and neutrophil count $\geq 1.5 \times 10^9$/L at entry; serum bilirubin ≤ 35 μmol/L; calculated creatinine clearance over 50 ml/min (Cockcroft and Gualt); and a life expectancy > 3 months. Information on prior radical surgery and specific sites of metastatic disease was not collected. Previous adjuvant chemotherapy following resection was allowed if completed >12 months previously. The estimated median (95% CI) survival of eligible patients was 7.1 (6.2, 7.8) months.[22]

Patients were excluded if they had radiotherapy within the previous four weeks. No other information on radiotherapy was collected as this was not used in the UK for locally advanced pancreatic cancer. Specific exclusion criteria were medical or psychiatric conditions compromising informed consent; intracerebral metastases or meningeal carcinomatosis; clinically significant serious disease or organ system disease not currently controlled on present therapy; uncontrolled angina pectoris; pregnancy or breast feeding;

previous chemotherapy for locally advanced and metastatic disease; radiotherapy within the last 4 weeks prior to start of study treatment; concurrent malignancies or invasive cancers diagnosed within the past 5 years except for adequately treated basal cell carcinoma of the skin, in situ carcinoma of the uterine cervix or resected pancreatic cancer; known malabsorption syndromes; hypersensitivity to any of the investigational products or patients with a dihydropyrimidine dehydrogenase deficiency; medication which might affect immunocompetence such as long term steroids or other immunosuppressants for an unrelated condition; men or women of reproductive potential, unless using at least two contraceptive precautions, one of which must be a condom.

The primary endpoint was to investigate the efficacy of GV1001 on length of survival when added concurrently or sequentially to the combination gemcitabine and capecitabine. The secondary endpoints were to evaluate the safety of GV1001 when added concurrently or sequentially to the combination gemcitabine and capecitabine; the efficacy measured as time to progression, objective response rate, quality of life, and changes in CA19-9 over time; immunogenicity measured as DTH and T-cell proliferation. The trial conformed to the principles of the International Conference on Harmonisation on Good Clinical Practice and was undertaken by the Cancer Research UK Liverpool Cancer Trials Unit; pharmacovigilance was sub-contracted to Orion Clinical Services Ltd (Slough, UK). All participants provided written, informed consent before randomisation.

Randomisation

Treatments were allocated with equal probability by means of computer-generated random permuted blocks of sizes 3 and 6 in equal proportion, employing the Stata add-in *ralloc*. Randomisation was stratified on stage of disease (locally advanced versus metastatic) and

ECOG performance status (0, 1 and 2). The allocation sequence was generated by the then Trial Statistician and was held centrally with access restricted to the TeloVacTrial Senior Statistician, the Senior Trial Co-ordinator and the Data Managers. Patients were randomised by trained authorised staff within the Liverpool Cancer Trials Unit.

Procedures

Haematology and serum chemistry was undertaken at screening baseline then weekly for the first three consecutive weeks out of every four weeks until the end of study. Computed tomography scans were performed at baseline, then at eight, 20 and 32 weeks after the start of therapy and thereafter 12 weekly and assessed using RECIST criteria. Quality of life was assessed at baseline, eight weeks, and then every 12 weeks after the initial scan at eight weeks using the EORTC QLQ-C30 questionnaire.

Patients randomised to control combination chemotherapy (arm1) received gemcitabine (1000mg/m^2 30 minute intravenous infusion on days one, eight and 15) and capecitabine (830 mg/m^2 orally twice daily of for 21 days) repeated every 28 days for six cycles or until disease progression, development of cumulative toxicities or patient choice.[22] (Supplementary Figure S1).

Patients randomised to sequential chemoimmunotherapy (arm 2) received two cycles ofcombination chemotherapy, and were then immunised by an intradermal injection of recombinant GM-CSF (75µg) into the lower abdomen and 10-15 minutes later an intradermal injection at the same site of 0.56 mg (200µl of 2·8 mg/ml) of GV1001.[10] The GV1001 vaccine was manufactured by Elaiapharm SAS (2881 Route des Crêtes, BP 205 Valbonne, 06904 Sophia Antipolis Cedex France). Immunisation was undertaken on days one, three, andfive during week one, then once on weeks two, three, four, andsix and then

monthly. The primary vaccination schedule was defined as the first 10 weeks of vaccination, 18 weeks after the start of chemotherapy. If there was disease progression GV1001 was stopped and gemcitabine and capecitabinewas re-started.

Patients randomised to concurrent chemoimmunotherapy(arm 3) received combination chemotherapy with GV1001 from day one of therapy. The primary vaccination schedule was similarly defined as the first 10 weeks of vaccination, but in this arm 10 weeks after the start of chemotherapy.

Dose reduction by 75% on the day of gemcitabine administration was required if the absolute neutrophil count was reduced to 0.5-1.0 $x10^9$/L and omitted for one week if $<0.5x10^9$/L ; following an episode of febrile neutropaenia, all subsequent courses would be given at 75% dose. Dose reduction by 75% on the day of gemcitabine administration was also required if the absolute platelet count was reduced to 50-100$x10^9$/L and omitted for one week if $<50x10^9$/L. Dose modifications of capecitabine for non-haematological toxicities notably gastrointestinal disorders, especially diarrhoea, nausea, vomiting and stomatitis, and hand-foot syndrome for grade 2 (NCI CTCAE) were to interrupt until resolved to grade 0-1 if first appearance and maintain dose level for the next cycle; if second appearance then interrupt and reduce to 75% with prophylaxis in the next cycle; and if third appearance interrupt and reduce 50% in the next cycle. Similarly for grade 3 toxicity for first and second appearances but discontinue treatment permanently if third appearance and also if grade 4. If patients experienced an episode of febrile neutropenia or had a neutrophil count of $<0.5x10^9$/L or aplatelet count < 50 $x10^9$/L, then capecitabine should be withheld and omitted until resolved to grade 0-1 toxicity. Patients should restart capecitabine at 75% dose.

DTH testing was undertaken in arms 2 and 3 by administering simultaneously a second intradermal injection of 0·105mg of GV1001 at the contra-lateral site on the lower abdomen without concomitant GM-CSF and read 48 hours later. DTH testing was continued until the result was positive or the vaccine therapy discontinued.

T-cell proliferation wasassessed in a sub-set of patients. Thawed peripheral blood mononuclear cells (PBMCs) were seeded in 48 well plates (ThermoFisher Scientific, USA) at $2x10^6$ cells/well in X-VIVO 15 (Lonza, UK) with 10% pooled human serum (Innovative Research, USA) and 20µg/ml GV1001 peptide. Following three days of culture, the media was changed to a final concentration of 10 units/ml of interleukin (IL)-2 (Peprotech, UK). On day 11, the GV1001-enriched cells were harvested and placed in a round bottom 96 well plate (50µl, $1x10^5$cells/well). To the pre-stimulated cells, irradiated (45Gy) autologous PBMCs (50µl, 1x 10^5cells/well) were added to act as antigen presenting cells. GV1001-specific proliferation was tested for by the addition of 100µl of either control media, 20µg/ml GV1001, or positive control with 5µg/ml phytohaemagglutinin (PHA). After afurther 48 hours incubation, 1µCi/well of ^{3}H-thymidine was added for 16 hours before counting. A positive proliferative response to GV1001 was defined as a stimulation index above 1·8 with a significant difference in counts per minute from four replicates. A positive total immune response was defined as a positive DTH test and/or a positive proliferation assay.

Patients could withdraw because of patient's decision to discontinue in the trial; disease progression according to RECIST; and intolerable adverse effects. Patients had to withdraw due to pregnancy; recurrent grade 3 or 4 drug related toxicity despite dose modification; serious systemic allergic reaction to any of the study drugs such as angio-oedema or

anaphylaxis; intercurrent, unrelated conditions requiring long term usage of steroids; and should they miss two consecutive GV1001 administrations or three GV1001 non-consecutive administrations during the entire treatment course and/or two consecutive cycles of gemcitabine and capecitabine administrations or three non-consecutive cycles during the entire treatment course. The National Cancer Institute Common Toxicity Criteria for Adverse Events versdion 3 was used to record toxicity

Statistical Analysis

1110 patients (780 deaths expected), were required to detect a 10% improvement in one-year survival foreither experimental arm above the 25% survival expected in the standard arm. A two-sided α of 0·025 (corresponding to a 97·5% confidence interval) was set for each primary response comparison of chemoimmunotherapy versus standard chemotherapy,with a total 0·05 level of significance andat least 80% power for the trial as a whole. The sample size additionally adjusted for four formal interim and one final analysis on the primary endpoint (O'Brien–Fleming type boundaries based on the Lan-DeMetsα-spending function). For the third and subsequent interim analyses futility for the primary outcome was assessed by estimating conditional power. In practice, as the trial terminated early, 98.25% intervals (significance of 0.0175) were applied to all analyses of the primary endpoint involving comparison between treatment arms.

The full analysis set consisted of all randomised patients in order to follow the intention to treat principle excepting for patients withdrawing consent between randomisation and starting therapy and patients withdrawn from the study after randomisation because of irregularities with the consent process. The per protocol set consisted of those patients in the full analysis set without any major protocol deviations. For per-protocol analysis for the primary endpoint, deviations were reviewed by the chief investigator to categorise protocol deviations

that would affect the outcome. The safety set comprised all patients who received any trial treatment. Sensitivity analysis included testing the proportional hazards assumption, additional stratification for CA19-9 levels, allowance for random centre effect and testing for treatment x centre interaction. For per-protocol analysis for the primary endpoint, deviations were reviewed by the chief investigator to categorise protocol deviations that would affect the outcome.

The final statistical analysis was performed with Stata v12.1, based on intention-to treat. Overall survival and time to progression were estimated by the Kaplan-Meier method, with hazard ratios (HRs) and confidence intervals obtained from a stratified Cox model and p-values from the corresponding stratified log-rank test (strata as defined for the randomisation). For other pre-specified outcomes (including time to progression), for descriptive analyses involving only a single arm and for unplanned analyses a conventional 95% two sided confidence interval was used with no correction.

The trial was sponsored by the Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, UK. The GM-SF and GV1001 vaccine were supplied by KAEL-GemVax Co.,Limited and the capecitabine was supplied by Roche.

The Protocol may be viewed on the following link:

https://www.lctu.org.uk/trial/trial_links.asp?id=42&tgcode=4&menuid=43

Role of the Funding Sources and Sponsor

Neither the sponsors nor funders had any role in the design and conduct of the study; the collection, management, analysis, and interpretation of the data; nor the preparation, review, or approval of the presentation. GM, PS, TC, WG, VS, EC, CR, GN and JN had access to the

raw data. The corresponding author had full access to all of the data and the final responsibility to submit for publication

RESULTS

There were 1572 patients who were screened to take part in the study with 402 excluded due to ineligibility and 108 declining to take part. The first patient was randomised on the 29th March 2007 and the trial was terminated slightly ahead of target on the 27th May 2011, with 1062 patients recruited, due to unfavourable survival fromsequential chemoimmunotherapy (CONSORT, Figure 1). Baseline characteristics were well balanced across all three groups (Table 1).

There were 741deaths, (95% of the 780 target number of deaths or 73% of the total). The 290 patients still alive had been followed up for a median of 6·0 (inter-quartile range,2·4-12·2) months. The overall median survival was 7·60 (95% CI, 7•11-8•12) months with 12 and 24 month survival rates of 30·43 (95% CI, 27·34-33·•56) % and 9·42 (95% CI, 7·23-11·96)% respectively.Baseline clinical stage, blood carbohydrate antigen (CA) 19-9 levels and ECOG performance status were all associated with survival (p< 0•001 for each factor on univariate analysis). After mutual adjustment, the respective hazard ratios (each compared against the lowest category of each variable) were: metastatic disease, 1·51 (95% CI: 1·27 – 1·79, P<0·001; ECOG level 1, 1·46 (95% CI: 1·23 - 1·73, P<0·001); ECOG level 2, 3·08 (95% CI: 2·40 - 3·96, P<0·001); and for CA199 2nd-4th quartiles, 1·33 (95% CI: 1·07 - 1·65, P=0·009), 1·53 (95% CI: 1·24 - 1·89, P<0·001), 2·41 (95% CI: 1·95 - 2·97, P<0·001) (Table 2; supplementary figures S2-S4).

The median overall survival times for the chemotherapy arm (n=358), sequential chemoimmunotherapy arm (n=350) and the concurrent chemoimmunotherapy arm (n=354) were 7.89 (95% CI: 7.07- 8.85), 6.94 (95% CI 6.35-7.6) and 8.36 (95% CI: 7.30 -9.74) months respectively (Table 3 and Figure 2). The corresponding hazard ratios for the

chemoimmunotherapy arms were 1.19 (98.25% CI: 0.97-1.48, p=0.047) and 1.05 (98.25% CI: 0.85-1.29, p=0.64), with an overall log-rank of χ^2_{2df}= 4.3; P=0•11). Given the predetermined two-sided α of 0·025 this was not statistically significant. The 12 month survival rates were 33·7%, 25·3% and 32·3% respectively. The lack of treatment effect on survival was consistent between the stages (Global Likelihood-ratio test for Treatment x Stage interaction, χ^2_{2df}= 1•36, P= 0•51).Compared to chemotherapy alone there was no survival benefit in patients with a lower tumour burden (locally advanced) and the best performance status (ECOG = 0) randomised to sequential chemoimmunotherapy (HR =1·20; 95% CI 0·59-2·45)nor to concurrent chemoimmunotherapy (HR=0·85; 95% CI 0·41-1·76) (Figure 2).An unplanned post-hoc analysis showed that the overall survival of the 84 patients randomised to sequential chemoimmunotherapy who returned to standard chemotherapy following progression on vaccination therapy and alive at 18 weeks was not dissimilar to patients alive at 18 weeks randomised to standard chemotherapy (HR=0·84; 95% CI, 0·58-1·23; p=0·28) (Supplementary figure S5).

The possibility that treatment effects might differ according to tumour stage, ECOG performance status and CA19-9 level was assessed by a likelihood ratio test nesting a main-effects only model within one including interactions (χ^2_{12df}=13·76, P = 0·32).

The median overalltimes to progression (TTP) for the chemotherapy, sequential chemoimmunotherapy and the concurrent chemoimmunotherapy arms were 6.35 (95% CI: 4.77- 7.07), 4.54 (95% CI 4.34-4.61) and 6.58 (95% CI: 5.03 -7.27) months, with corresponding hazard ratios for the chemoimmunotherapy arms of 1.50 (98.25% CI: 1.26-1.78, p<0.001) and 1.0 (98.25% CI: 0.84-1.19, p=0.99) with overall log-rank χ^2_{2df} = 29·5; P<0•001) (Figure 3).

A partial or complete clinical response was observed at eight weeks in 90 (8·47%) patients (95% CI, 6·80-10·15%) with no significant difference across the three arms. An overall response was observed in 149 (14·03%)patients(95% CI,11·94-16·12), significantly worse for patients randomised to sequential chemoimmunotherapy (Supplementary Table).

DTH waspositive in 19(12·3%) of 154 patients randomised to sequential immunotherapy (conditional on survival to 18 weeks) and in 47 (20·2%) of 233 patients randomised to concurrent chemoimmunotherapy(conditional on survival to 10 weeks) (P=0·0531). Patients on sequential immunotherapy with a positive DTH response had a median survival of 7·5 (95% CI, 3·5-9·5) months and 5·8 (95% CI, 3·9-7·1) months with a negative response (HR=0·95; 95% CI, 0·49-1·84) (χ^2_{1df}=0·036, P=0·85). The median survival in patients onconcurrent chemoimmunotherapywith a positive DTH response was 9·0 (95% CI, 6·1-10·9) months and 8·0 (95% CI, 6·6-8·7) months with a negative response(HR=0·98; 95% CI, 0·60-1·59) (χ^2_{1df} = 0·015, P=0·90) (Figure 4).

T-cell proliferation waspositive in 10 (31·3%) of 32 patients given sequential immunotherapy and 10 (14·7%) of 68 patients givenconcurrent chemoimmunotherapy(P=0·065). There was a total immune response in 12 (37·5%) of32 patients on sequential immunotherapy and 25(36·8%) of 68 patients onconcurrent chemoimmunotherapy (P=1•0); 12 (40%) of 30 and 25 (39.6%) of 63 patients respectively without missing values (p=1•0).The median survival (from week 18) in patients on sequential immunotherapy arm witha positive total immune response was 8·4 (95% CI, 7·0-NA) months and 7·3 (95% CI, 3·0-29·2) months with a negative response (HR=0·28; 95% CI, 0·05-1·53)(χ^2_{1df}=2·368, P=0·12). The median survival (from week 10) in patients randomised to concurrent chemoimmunotherapy was 10·6 (95%

CI, 6·2-13·7) months and 12·2 (95% CI, 7·2-16·6) months respectively (HR=1·57; 95% CI 0·71-3·49) (χ^2_{1df} =1·241, P = 0·27) (Supplementary figure S6). The pooled hazard ratio for positive total immune responsewas 1·15 (95%CI, 0·56-2·38) (χ^2_{1df} =3·208, P=0·073).

The standard chemotherapy regimen was well tolerated and there was no evidence of any additional toxicity from sequential or concurrent immunotherapy (Tables 4a nd 4b). The numbers of patients with dose modification as a result of toxicity in each arm are shown in table 4c. There were 32 (3%) patients altogether who withdrew because of toxicity, 15 (4%) patients from the standard chemotherapy arm, four (1%) from the sequential chemoimmunotherapy arm and 13 (4%) from the concurrent chemoimmunotherapy arm (χ^2_{2df}=6.4; p=0.04). There were four (1%) deaths due to drug-related toxicity in the standard chemotherapy arm, five (1%) in the sequential chemoimmunotherapy arm and six (2%) in the concurrent chemoimmunotherapyy arm (P=0·811). Patients randomised to sequential immunotherapy had a significantly higher pain score compared to patients randomised to standard chemotherapy at 20 weeks (fitted mean difference 32·88; 95% CI, 17·49-48·26; P<0·001) and a lower QOL score (fitted mean difference -19·62; 95% CI, -34·90- -4·34;P=0·012) (Table 5).

The per protocol analysis was undertaken after removing 62 deviations (21, 21 and 20 in Arms 1, 2 and 3 respectively) that were identified as having the degree of seriousness deserving exclusion. The results of this analysis did not materially alter in any way the outcomes of the intention to treat analysis.

DISCUSSION

The trial demonstrated that there was no survival benefit for the addition of the GV1001 vaccine to gemcitabine and capecitabinein patients with advanced pancreatic cancer. A positive DTH, T-cell proliferation and total immune response was observed in 12.3%, 31·3%and 37·5% respectively of patients given sequential chemoimmunotherapy and in 20.2%, 14.7% and 36·8% respectively of patients given concurrent chemoimmunotherapy but there was no association with survival. The total immune response in the phase II study compared to that seen in the current study is consistent in the reduction in response rates generally observed in the transit from phase II to phase III studies.[10] In order for a cancer vaccine to be effective an active immune response is needed and this is dependent on a sufficient period of time for this to develop. The characteristic early metastasising and rapidly progressive nature of pancreatic cancer may partly explain the lack of clinical efficacy but there are also other complex immunological and stromal factors that need to be overcome.[24-26]The dense stromal reaction impedes the penetration of cytotoxics into pancreatic tumours thus limiting the synergistic potential of chemotherapy and GV1001 vaccination intended to achieve CD40 activation and generate telomerase-specific T helper cells.[25] Direct CD40 activation (using the agonist CP-870,893) with gemcitabine chemotherapy has recently been shown to cause a partial tumour response in four of 21 patients with advanced pancreatic cancer but surprisingly this effect was due to stroma infiltrating macrophages rather than T cells.[26]

An effective active immune response may also be dependent on a lower tumour burden and good performance status but recent studies from other tumour typesdo not appear to support this. In a randomized phase II trial of prostate cancer involving PROSTVAC-VF (consisting of two prostate specific antigen [PSA] encoding viral vectors, and the B7.1, ICAM-1, and

LFA-3 co-stimulatory molecules) and GM-CSF there was no survival improvement in men with fewer bony metastases or an ECOG performance status of zero.[27]Similar findings were found in the IMPACT phase III trial in men treated with sipuleucel-T (Provenge) a form of active cellular immunotherapy(autologous PBMCs activated with the recombinantPA2024 protein composed of a prostate antigen and prostatic acid phosphatase, fused to GM-CSF).[28] In the TeloVac trial there was also no impact on survival by either stage of disease or performance status from GV1001 vaccination.

Identifying robust immunotherapy response signatures in human cancer studies remains challenging across tumour types.In the PROSTVAC-VF trial there was no antibody response to PSA and although all generated titres to one or both viralvectors there was no association with survival.[27]In the IMPACT trial, although there was a T-cell proliferation response to PA2024 in 46 (73·0%) of 63 patients given sipuleucel-T, only 15 (27·3%) of 55 patients had a T-cell response against the target antigen prostatic acid phosphatase. As in the TeloVac trial there was no association with survival although patients with a high antibody titre against PA2024 lived longer.[28]The limited immunotherapeutic responses seen in these studies as well as in TeloVac trial may partly be due to inhibitory T-cell checkpoints that minimise T-cell cytotoxicity.[5] These include the cytotoxic T-lymphocyte associated antigen (CTLA)-4 binding of ligands and at a later point programmed death (PD)-1 receptor activation.[5]Combined blockade of both these check points produced a tumour response in 21 (40%) of 52 patients with advanced melanoma.[6]A randomised phase II study of 30 patients previously treated for advanced pancreatic cancer comparing the anti-CTLA-4 antibody ipilimumab alone with ipilimumab plus a GM-CSF cell-based vaccine (GVAX) showed stable disease in two patients in each of the two arms with no objective responses although there was a trend towards increased median survival of 5.7versus 3.6 moths respectively.[29]

In a trial of an anti-PD-L1 antibody (BMS-936559) in 207 patients, objective responses were reported in malignant melanoma, renal-cell cancer, non-small cell lung cancer, and ovarian cancer but none in 14 patients with advanced pancreatic cancer.[30] The relative lack of immunotherapy efficacy in pancreatic cancer may also in part be related to specific carcinoma-associated fibroblasts (expressing fibroblast activation protein) which secrete CXCL12 and thus stop T cells from accessing cancer cell regions in the stroma. In a genetically engineered mouse model of pancreatic cancer blocking the receptor of CXCL12, induced rapid T-cell accumulation and synergised with α-PD-L1 in cancer cell killing.[31]

There have been concerns raised about the use of GM-CSF as adjuvantbased on the induction of myeloid derived suppressor cells (MDSCs) by low-dose GM-CSF,[32] but we have demonstrated that the levels of MDSCs were reduced in patients treated with GV1001, GM-CSF and concomitant chemotherapy compared with chemotherapy alone.[33]

There was a shorter time to progression observed after the administration of two cycles of chemotherapy in the sequential chemoimmunotherapy arm. A post-hoc analysis showed that the overall survival in this group of patients who returned to standard chemotherapy following progression on vaccination therapy was not different from comparable patients randomised to standard chemotherapy. These findings suggest that treatment until progression is the appropriate approach in pancreatic cancer and is consistent with the recent findings that a minimum of six cycles of chemotherapy is necessary for optimum survival benefit in the adjuvant setting.[34] The TeloVac study has demonstrated the challenges for immunotherapy study design when immunotherapy is combined with standard therapy in the first line setting and highlights the uncertainties in extrapolating treatment scheduling from experimental models to the clinical setting. One option that had been considered was

vaccination after six cycles of chemotherapy rather than two cycles but it was important to test institution of vaccination after initial stabilization of disease before progression, and at a time when apoptosis was likely to be still high. In this study the median progression free survival on chemotherapy alone was 6.3 months therefore 50% of patients would have progressed at this point. For future immunotherapy trials randomized phase II studies employing adaptive trial designs should be seriously considered to explore different scheduling regimens that do not compromise standard treatment.

The TeloVac trial has demonstrated that vaccination to hTERT can elicit immune responses during chemotherapy but without clinical efficacy. There are presently a number of different approaches being used to target telomerase in cancer including small-molecule telomerase inhibitors which may be more effective in tumours with shorter telomeres.[35, 36] There are opportunities now to uncloak the mechanism of action of vaccines such as GV1001 using multi-modality strategies that are directed against the stroma and check point inhibition as well as direct CD40 activation.

PANEL: RESEARCH IN CONTEXT

Systematic review

In trial set up for the treatment of locally advanced and metastatic pancreatic two systematic reviews were undertaken.[37,38] The search (Medline, OLDMEDLINE, CancerLit,EMBASE, and ISI Web of Science, ISI Science and Technology Proceedings, Current contents databases, trial registries and conference proceedings) involved randomized controlled trials involving patients with advanced pancreatic cancer of chemotherapy, novel agents, radiotherapy, chemoradiotherapy and best supportive care. PubMed was searched for any

clinical trial and experimental work for telomerase, immunotherapy, vaccines, cancer, pancreatic cancer, and GV1001.[39]

The reviews showed that chemoradiation followed by chemotherapy did not demonstrate any survival advantage over chemotherapy alone. There was a significant survival benefit for chemotherapy over best supportive care and gemcitabine combinations over gemcitabine alone including gemcitabine with capecitabine. The evidence did not support the use of GV1001 vaccine alone but should be tested along with chemotherapy. The combination of gemcitabine with capecitabine was chosen as this could potentiate the vaccine effects and was also associated with significantly improved objective responses compared to gemcitabine alone.[22,37,38]

Interpretation

This is one of the largest randomized clinical trials ever undertaken in pancreatic cancer and the largest trial of vaccine therapy ever undertaken in any solid cancer type. There was no survival benefit from the addition of the GV1001 vaccine to gemcitabine and capecitabine in patients with advanced pancreatic cancer. Survival was not affected by immune response, stage of disease or performance status in patients given GV1001 vaccination.

The TeloVac trial demonstrated immune responses in patients being given a cancer vaccine with concurrent chemotherapy in a broadly similar proportion of patients receiving vaccine therapy alone in pancreatic and prostate cancer and also malignant melanoma. The TeloVac trial demonstrated progression of disease once the initial two cycles of chemotherapy was stopped, in the sequential chemoimmunotherapy arm, suggesting that treatment, including chemotherapy should continue until progression.

During the course of the trial there has been a major development in clinically relevant genetically engineered models that has significantly contributed to the deeper understanding of the biological mechanisms that undermine the effective treatment of pancreatic cancer.[25,26,31] These can now be countered and could be deployed in multimodality strategies to overcome the key biological constraints to effective treatment.

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Conflicts Of Interest

Drs **Anthoney, Archer, Corrie, Coxon, Eatock, Evans, Falk, Garcia-Alonso, Harrison, Hickish, Iveson, Madhusan, Nicoloson , Propper, Robinson, Roques, Steward** and **Wadd** report other from Roche , during the conduct of the study; Dr. **Wadsley** reports other from Roche, during the conduct of the study; personal fees from Astrazeneca, personal fees from Celgene, from Novartis, outside the submitted work; Dr. **Ross** reports other from Roche , during the conduct of the study; personal fees from Roche, grants and personal fees from Merck Serono, personal fees from Cellgene, personal fees from Bayer, grants and personal fees from Sanofi Aventis, personal fees from Novartis, personal fees from Bristol Myers Squibb, personal fees from SIRTex, outside the submitted work; .Dr **McAdam** sits on chair of meeting on ER +ve metastatic breast cancer for Astra Zeneca and advisory board on denosumab for AMGEN; Dr. **Middleton** reports other from Roche , grants from Cancer Research UK, grants and personal fees from KAEL Gemvax, during the conduct of the study; .Dr. **Neoptolemos** reports other from Roche , grants from Cancer Research UK, grants and personal fees from KAEL Gemvax, during the conduct of the study; personal fees from Oxford Biomedica (UK) Ltd, personal fees from Pzifer Novartis, personal fees from Astellas, personal fees from Novartis Pharma AG, outside the submitted work; and Professor John Neoptolemos is an NIHR Senior Investigator and is part funded by the NIHR Biomedical Research Centre at the Royal Liverpool University, Liverpool; Drs. **Costello, Cox, Greenhalf, Naisbitt, Nanson, Rawcliffe, Silcocks** and **Shaw** report other from Roche grants from Cancer Research UK, grants and personal fees from KAEL Gemvax, during the conduct of the study; Dr. **Valle** reports other from Roche , during the conduct of the study; personal fees from Lilly Oncology, outside the submitted work; Dr. **Cunningham** reports other from Roche , during the conduct of the study; personal fees from Roche, personal fees from Amgen, personal fees from Celgen, personal fees from Sanofi, personal fees from Merck Serono, personal fees from Novartis, personal fees from AZ, outside the submitted work; and Professor David Cunningham is an NIHR Senior Investigator and is part funded by the NIHR Biomedical Research Centre at the Royal Marsden Hospital; No other authors declared any conflict of interest.

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Author Contributions

The Academic Chief Investigator Professor Gary Middleton and the principal grant holder Professor John P Neoptolemos had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.

All of the authors gave final approval of the version to be published. Development of the study design was supported and conducted through National Cancer Research Institute (NCRI) of the UK Pancreatic Cancer Sub-Group and the CRUK Liverpool Cancer Trials Unit(of the Liverpool Clinical Trials Unit) and was led by the TeloVac working party comprising G Middleton, J Neoptolemos, D Cunningham, T Cox, P Silcocks, J Valle, J Wadsley, D Propper, F Coxon, P Ross, S Madhusudan, T Roques and P Corrie. P Silcocks and Trevor Cox were responsible for detailed statistical analysis. Drs W Greenhalf, V Shaw, E Costello and D Naisbitt developed and were responsible for the translational aspects of the trial. Charlotte L. Rawcliffe and Gemma Nanson were the senior trial coordinators

responsible for central administration ensuring ethical standards for collection and verification of data. The results were interpreted by theTeloVac working party, which prepared the initial draft and were responsible for collating changes proposed by all of the authors into the final draft paper before final approval by all participants TeloVac Study Group.

Participants in theTeloVac Trial

The specialists who also contributed to the recruitment, treatment and follow-up of patients as trial site Principle Investigators, along with sites and number of patienst recruited are shown in the on line appendix.

LEGENDS

Figure 1. CONSORT diagram

Figure 2.Overall survival by treatment arm and hazard ratios of sequential chemoimmunotherapy (arm 2) versus standard chemotherapy (arm 1) and concurrent chemoimmunotherapy (arm 3) versus standard chemotherapy (arm 1) by ECOG performance status and stage.

Figure 3.Time to progression by treatment arm.

Figure 4.Overall survival according to a positive or negative DTH response in patients randomised to sequential chemoimmunotherapy (arm 2) conditional on reaching week 18 (A) and in patients randomised to concurrent chemoimmunotherapy (arm 3) conditional on reaching week 10 (B).

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